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Publication date:
2012

Document Version
Publisher's PDF, also known as Version of record

Metabolomics of UC bacterial ecosystem compared to the healthy donors.

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Introduction

Ulcerative colitis[1] (UC) is an idiopathic inflammatory bowel disease (IBD), which is characterized by chronic inflammation of the colonic mucosa. As the etiology of IBDs remains still unknown, it has been shown in many studies that patients with UC have an altered bacterial microbiota[2]. Thus, the bacterial and/or host-bacterial interactions may play role in the pathogenesis of UC. Communication between them is a very complex system, based on fx. enzymes, DNA, proteins and small molecules exchange. This study focus on the metabolic interactions, corresponding to the described bacterial microflora by qPCR. The aim of this study was to examine the difference in the metabolites profile of fecal microbiota derived from UC patients and healthy subjects, colonizing a dynamic *in vitro* gut model.

Materials and methods

Fecal samples came from 4 healthy volunteers and 8 UC patients (4 in remission and 4 in relapse state). A dynamic *in vitro* model, the M-SHIME[3], was set up to simulate stomach, small intestine and 6 colon vessels, which were run in parallel (Fig.1). Mucin-covered microsms were incorporated into the luminal suspension of all colon vessels. Each colon compartment was inoculated with a microflora from one, specific donor. SHIME feed was distributed into all types of vessels. Pancreatic juice was added to the stomach and small intestine parts. To handle all of the donors, system was run 2 times[2]. For metabolic studies mucus and lumen samples were taken from the M-SHIME after 42 hours. In order to extract metabolites cold MeOH was used. Metabolites were detected by LCMS as follow: a Dionex Ultimate 3000 RS liquid chromatigraph coupled to a Bruker maXis time of flight mass spectrometer. Analytes were separated on a Kinetex PFP column 50 x 2.10 mm, 2.6 µm, 100Å, using solvents: 10 mM NH_4HCO_2 and C_2H_3N as a linear gradient from 0 to 90% C_2H_3N over 8 min. Scan range was from 50 to 800 m/z. Samples were analyzed in negative mode (due to the high amount of TWEEN 80 in the samples positive mode was disregarded). The differences in metabolite profiles[4] were evaluated by principal component analysis (PCA) using Profile Analysis 2.0 by Bruker Daltonics.

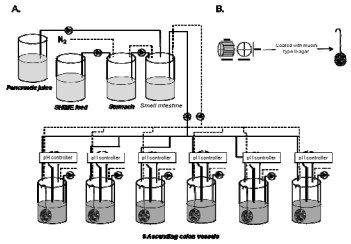

Fig.1. A. Schematic overview of the M-SHIME. **B.** Detailed scheme of mucin microcosms.

Results and discussion

PCA (Fig.2) shows a distinctive separation between UC in relapse and healthy donors for the mucus samples, which confirmed the data, describing bacterial differences between two types of microflora, made for the same samples. The same result was observed for the lumen samples. Metabolites separating those two groups are bile acids, fatty acids, drug and drug metabolites present in the UC patients group. It also seems like tryptophan metabolism is different in the healthy community then in the UC – this amino acid can be seen in the healthy group and only possible derivatives from it, in the UC. Small differences between metabolites in mucus and in the lumen were also present. These results will be further studied and combined with the qPCR data, describing the changes in the bacterial community for the healthy donors and UC patients.

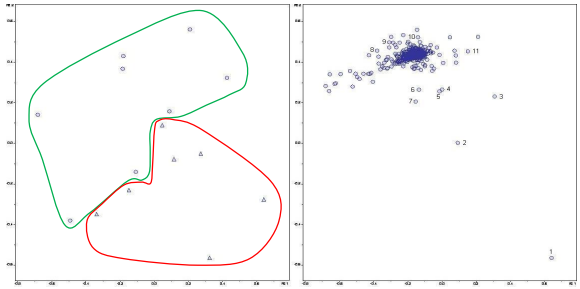

Fig.2. PCA plot from metabolite analyses based on the mucus samples from UC (Δ) and healthy donors (○). Scores plot on the left, loading plot on the right. PC1(26%) vs PC2(17%).

Acknowledgments

This work was supported by the Danish Council for Strategic Research, Technical University of Denmark, Øresund Food and a GOA project from Ghent University.

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